

## Mini-preparation of plasmid DNA by alkaline lysis

Unit 1.6, Ausubel et al. 1999 (NUF 00.5.2; modified j.a.m. 07/07)

1. Inoculate 1.5-5 ml LB amended with appropriate antibiotic(s) with a colony taken from a selective plate. Grow until late exponential phase (until dense), 7-12 hours depending on antibiotic and growth temperature.
2. Microcentrifuge 1.5 ml of cell culture for 30 s – 1 min. in an eppendorf tube and remove supernatant. The pellet may be stored at -20°C.
3. Resuspend the pellet in 100 µl of Solution I by vigorous vortexing (make sure no clumps of cells are visible) and let stand for 5 min. at room temperature.
4. Add 200 µl lysis solution (Solution II), mix gently (by inversion), and incubate for 10 min. at room temperature.
5. Add 150 µl of potassium acetate solution (Solution III), vortex briefly, and incubate 10 min. on ice.
6. Microcentrifuge for 4 min. at room temperature and transfer the supernatant to a fresh tube.
7. Add 2-4 µl of 10 mg/ml RNase A and incubate at 37°C for 30-45 min.
8. Add 400 µl chloroform-isoamyl alcohol (24:1 by vol) and vortex. Microcentrifuge for 5 min. at room temperature and transfer the upper (aqueous) phase to a fresh tube.
9. Add 800 µl of 95% ethanol or 400 µl isopropanol, vortex, and microcentrifuge for 10 min at room temperature. Discard the supernatant.
10. Wash pellet with 700 µl of 70% ethanol (do not resuspend), microcentrifuge 3 min. at room temperature, and discard the supernatant.
11. Dry the DNA.
12. Resuspend in 20-50 µl TE or ddH<sub>2</sub>O.

The typical yield of high-copy-number plasmids such as pUC is about 3-5 µg DNA per ml of original cell culture.

### Birnboim & Doly I

50 mM glucose  
25 mM Tris-HCl  
10 mM EDTA  
pH 8.0

Autoclave and store at room temperature.

### Birnboim & Doly II

Prepare 2 solutions:

0.4 M NaOH

2% w/v SDS

Store at room temperature.  
Mix 1:1 immediately before use.

### Birnboim & Doly III

294 g potassium acetate (3M)  
50 ml 90% formic acid OR  
115 ml glacial acetic acid.  
ddH<sub>2</sub>O to 1 L  
pH should be ~5.5

Store at room temperature or at 4°C.